A Cytokine Profile in Response to Stimulation of Peripheral Blood Mononuclear Cells by HCV C200

Thesis

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List of Abbreviations

aa : amino acid
Ag : Antigen
ALT : Alanine aminotransferase
APCs : Antigen presenting cells
ARF : Alternate reading frame
ARFP : Alternate Reading Frame Protein
AST : Aspartate transaminase
bDNA : Branched DNA
CD : Cluster of differentiation
CDC : Centers for disease control and prevention
CFSE : Carboxyfluorescein diacetate succinimidyl ester
CMI : Cell mediated immunity
CTL : cytotoxic T lymphocytes
D : Domain
DCs : Dendritic Cells
DNA : deoxyribonucleic acid
dsRNA : Double stranded RNA
E : Envelope glycoprotein
EIA : Enzyme immunoassay
ELISA : Enzyme-Linked Immunosorbent Assay
ELISPOT : Enzyme-linked immunospot
ER : Endoplasmic reticulum
F : Frameshift
HCC : Hepatocellular carcinoma
HCV : Hepatitis C Virus
HCWs : Health care workers
HLA : Human leukocyte antigens
HS : Highly Significant
HVR : Hypervariable region
IFNI : Type I interferon
IFN-α : Interferon alpha
IFN-β : Interferon beta
IFN-γ : Interferon-gamma
IgG : Immunoglobulin G

IKK : Inhibitor of kB kinase
IL-2 : Interleukine-2
IL-2R : IL-2 receptors
IPS-1 : IFN-β promoter stimulator protein 1
IRES : Internal ribosomal entry site
IRF : Interferon regulatory factor
ISDR : IFN-α sensitivity determining region
ISGF : IFN stimulated gene factor
ISGs : Interferone stimulation genes
ISREs : IFN-stimulated response elements
JAK : Janus tyrosine kinase
KIR : Killer inhibitory receptors
LFTs : Liver Function Tests
MDCs : Myeloid DCs
MHC : Major histocompatibility complex
MICA/B : MHC class-I related chain A/B
NK : Natural killer cells
NKT : Natural killer T cells
NS : Non Significant
NS proteins : non structural proteins
NTPase : Nucleoside triphosphatase activities,
PBMCs : Peripheral blood mononuclear cells
PCR : Polymerase chain reaction
PD-1 : Programmed death 1
PDCs : Plasmacytoid DCs
PD-L : programmed death ligand
PI : Proliferation index
PIAS : Protein inhibitor of activated STAT1
PKR : dsRNA-dependent Protein Kinase R
PP2A : Protein phosphatase 2A
QPCR : Quantitative PCR
RdRp : RNA-dependent RNA polymerase
RFU : relative fluorescence units
RIBA : Recombinant immunoblot assay
RIG-I : Retinoic acid–inducible gene I
RNA : Ribonucleic acid
RT : Reverse Transcriptase

S : Significant

Sig : Significant

SOCS : Suppressor of cytokine signaling

SP : Signal peptidase

SPSS : Statistical Package for Social Sciences

SR-BI : Scavenger receptor B type I

STAT : Signal transducer and activator of transcription

T regs : Regulatory T cells

TBK1 : TNF receptor– associated factor family member–associated NF-κB activator–binding kinase–1

TCR : T cell receptor

Th cells : T helper cells

TLR : Toll like receptor

TMA : Transcription mediated amplification

TMD : Transmembrane domain

TNF-α : Tumor necrosis factor-alfa

TRIF : Toll–IL-1 receptor domain–containing adaptor inducing IFN-β NF-κB nuclear factor kappa B

TYK2 : Tyrosine kinase 2

UTR : Untranslated regions

γGT : Gamma Glutamyl Transferase

2′-5′ OAS : 2′-5′Oligoadenylate synthetase

5-PL : Five-Parameter Logistic curve

Introduction

Hepatitis C virus (HCV) infection has become a global health problem with around 170–190 million infected people worldwide ***(Berenguer, 2007).***

Spontaneous viral clearance of HCV is observed in only 15–40% of patients with acute hepatitis whereas, persistent infection is established despite evidence of immune regulation and is associated with progression to cirrhosis and hepatocellular Carcinoma ***(Shiffman, 2003).***

Hepatitis C virus has structural proteins like core protein and envelope glycoproteins E1 and E2, which mediate entry into cell by binding to CD81 as well as the non-structural (NS) proteins, which have essential functions in viral replication. NS3 contains protease, RNA helicase and nucleoside triphosphatase (NTPase) activities, all of which are essential to viral replication. NS4A acts as cofactor for N3 protease activity. NS4B has a role in the formation of HCV RNA replication complex ***(Inoue et al., 2007).***

Strong and persistent cell mediated immune responses have been reported in HCV seronegative individuals with documented exposure to HCV in the absence of detectable viral *RNA* ***(Post et al., 2004).***

HCV-specific $CD4^+$T cell responses persist in acutely infected individuals who permanently cleared the virus, and disappear in patients whose viraemia subsequently recurred ***(Chang et al., 2001).***

All patients with self limited disease had a significant $CD4_+$ T-cell proliferation to HCV specific C200 peptide, running parallel with the antigen-stimulated secretion of IL-2 and IFN- γ but not with IL-4 and IL-10, indicating predominant Th1 response ***(Semmo and* Klenerman*, 2007).***

HCV-specific $CD8^+$T cell responses that are associated with spontaneous viral clearance tend to be multi-specific and polyclonal ***(Lauer et al., 2005).***

Because of the high variability of HCV, escape mutations in $CD8^+$T cell epitopes are common and they are expected to play a major role in chronic viral persistence ***(Ray et al., 2005).***

HCV-specific $CD8^+$T cells have both cytolytic and non cytolytic effector function. The non cytolytic function is mediated by production of Interferone gamma ***(Guidotti et al., 2001).***

The imbalance between T helper cell Th1 (IL-2, IL-12, IFN-γ) cytokines and Th2 (IL-10) cytokines affect the outcome of HCV infection. The defect in both IL-12 and IFN-γ production may contribute to persistence of HCV infection ***(Sarih et al., 2000).***

IL-2 was capable of both pushing semi-effector CTL to complete its effector cell program and restoring the HCV core-dependent inhibitory effect ***(Accapezzato et al., 2004).***

TNF-α triggers a partially overlapping set of antiviral defense mechanisms and serum level of TNF-α reflects the progression of inflammation ***(Akyűz et al., 2005).***

Interferon- gamma (IFN-γ) is a key cytokine and can inhibit HCV replication ***(Lanford et al., 2003).***

Aim of the work

The aim of the work is to detect a panel of cytokines IL2, IFN-γ and TNF-α of cell culture supernatant from unstimulated and stimulated peripheral mononuclear cells (PBMCs) by HCV specific C200 peptide.